ROLE OF DIFFUSION IN LIGAND BINDING TO MACROMOLECULES AND CELL-BOUND RECEPTORS

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ABSTRACT The association and dissociation rates of partially diffusion-controlled bimolecular reactions are considered. A simple expression for the equilibrium constant is derived using classical statistical mechanics. The relationship is established between the Collins-Kimball treatment, which is based on the "radiation" boundary condition involving an intrinsic rate constant κ, and the kinetic scheme $A + B \rightleftharpoons A\cdots B \rightleftharpoons AB$ where $A\cdots B$ is an encounter complex. It is shown that with the appropriate choice of the interaction potential, Debye's expression for the association rate constant becomes identical to that obtained using the radiation boundary condition if κ is evaluated using Kramers' theory of diffusive barrier crossing. Finally, the competitive binding of ligand to a spherical cell, whose surface is partially covered by multiple reactive sites, is studied by treating the cell as a partially reacting sphere.

INTRODUCTION

The diffusive motion of the reactants can play an important role in determining the rates of bimolecular reactions, such as ligand binding to macromolecules or cell-bound receptors and the self-assembly of multisubunit proteins. Although the rates of such reactions are usually fast, they are, with rare exceptions, not entirely limited by diffusion. This paper deals with the theory of such partially diffusion-controlled reactions (i.e., not every diffusive encounter results in the formation of a product). We consider both steady-state association and dissociation rate constants and clarify the physical basis of and the interelationship among a variety of approaches.

The classic work of Smoluchowski (1) calculates the association rate constant for the purely diffusion-controlled reaction of two isotropically reactive spheres, A and B, as follows. A single molecule of A is placed at the origin and the steady-state diffusion equation for the concentration of B [denoted by $c(r)$] is solved subject to the absorbing boundary condition that

$$c(R) = 0 \tag{1}$$

where R is the sum of the radii of A and B. In addition, one requires that as $r \to \infty$ $c(r) \to c_0$ where c_0 is the bulk concentration of B. The diffusion-controlled rate constant is then obtained from the flux at $r = R$ as

$$k_S = 4\pi DR^2 c'(R)/c_0 = 4\pi DR^2 \left(\frac{\partial c(r)}{\partial r}\right)_{r=R} \Big/ c_0 = 4\pi DR \tag{2}$$

where $D = D_A + D_B$. Debye (2) has generalized the above result for the situation that the reactants interact via a potential of mean force $V(r)$.

The Smoluchowski theory can also be extended to handle species that are not uniformly reactive over their surface (3–7). For example, for the reaction of a point ligand with a circular reactive site of radius a lying on an infinite plane, the association constant is (6, 8)

$$k_{ass} = 4Da. \tag{3}$$

Recently, we studied (7) the binding of a ligand to a macromolecule of radius R that has a single reactive site of radius a on its otherwise inert surface. We showed that when a/R is small, Eq. 3 is a good approximation to the rate, and noted that this rate is considerably larger than that obtained simply by multiplying k_S in Eq. 2 by the reactive fraction of the macromolecular surface area. Most of this paper deals with uniformly reactive species.

Collins and Kimball (9) generalized the Smoluchowski theory to partially diffusion-controlled reactions by replacing the perfectly absorbing boundary condition of Eq. 1 by the "radiation" (or partially absorbing) boundary condition

$$4\pi R^2 Dc'(R) = \kappa c(R), \tag{4}$$

i.e., the flux at contact is assumed to be proportional to the local reactant concentration. The constant of proportionality, κ, is an intrinsic rate constant whose value determines the extent of diffusion control.(When $\kappa = 0$ no reaction occurs, but when $\kappa \to \infty$ the reaction is completely diffusion limited.) The physical meaning of κ will be discussed in more detail later. It is of interest to note (see for example reference 10) that solving the steady-state diffusion equa-

tion subject to the above boundary condition is equivalent to using the following steady-state reaction-diffusion equation

$$D\nabla^2 c - \frac{\kappa}{4\pi R^2}\delta(r - R)c = 0 \tag{5}$$

where $\delta(r)$ is the Dirac delta function. The resulting association rate constant is (9)

$$k_{CK}^{-1} = \kappa^{-1} + k_S^{-1} \tag{6}$$

where k_S is given by Eq. 2.

One of the aims of this paper is to establish the relationship of the above-mentioned theories to the following (11, 12) two-step kinetic scheme

$$A + B \underset{k_-}{\overset{k_+}{\rightleftharpoons}} A\cdots B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB. \tag{7}$$

In this scheme, the first step is the formation of an encounter complex, A···B. The second step is the reaction itself. Making the steady-state approximation for the concentration of the complex, the effective forward and reverse rate constants (11, 12), k_f and k_r, are

$$k_f = \frac{k_+ k_1}{k_1 + k_-} \tag{8}$$

and

$$k_r = \frac{k_{-1}k_-}{k_1 + k_-}. \tag{9}$$

The equilibrium constant is

$$K_{eq} = \frac{k_f}{k_r} = \frac{k_+ k_1}{k_- k_{-1}}. \tag{10}$$

In this paper, we will relate the rate constants in the scheme of Eq. 7 to the diffusion-controlled association rate constant, to the Collins-Kimball intrinsic rate constant, κ, and to the capture or escape probabilities. Expressions will also be obtained for k_r and K_{eq}.

The outline of this paper is as follows: first, we summarize the expressions for the association constants obtained using both the Smoluchowski and radiation boundary conditions, and discuss the relationship of these results to first passage times (13). We then review the calculation of escape or capture probabilities using the approach of Onsager (14) and Tachiya (15). Next, we consider the relationship of these results to the kinetic scheme of Eq. 7. We discuss the physical significance of the Collins-Kimball intrinsic rate constant, κ, and obtain expressions for the dissociation rate constant. We then derive an exact expression for the equilibrium constant using classical statistical mechanics. Next, we show that for an appropriate choice of the interaction potential, the theory of Debye (2) gives a rate that is equivalent to that of Collins and Kimball (9). Moreover, the resulting expression for κ is identical to that obtained from Kramers' (16) theory of diffusive barrier crossing.

Finally, as an application, we consider the kinetics of ligand binding to a spherical cell containing a large number of receptors on its surface. Our approach to this problem is based on the observation that a cell which is partially covered with reacting sites can be regarded as a partially reactive sphere. By making a simple choice of the Collins-Kimball κ we immediately recover the results of Berg and Purcell (8) and those of DeLisi and Wiegel (17), which were obtained using different approaches.

ASSOCIATION RATE CONSTANTS

We consider two spherical, isotropically reactive species that interact via the potential of mean force, $V(r)$. To obtain the generalization of the Smoluchowski rate constant k_S (Eq. 2), we proceed as before but use the steady-state Smoluchowski equation, i.e.,

$$\nabla \cdot e^{-\beta V}\mathbf{D} \cdot \nabla e^{\beta V} c = 0 \tag{11}$$

where $\beta = (k_B T)^{-1}$ instead of the free diffusion equation. Solving this equation subject to boundary condition of Eq. 1 and calculating the flux as in Eq. 2, one has

$$k_{SD}^{-1} = (4\pi)^{-1}\int_R^\infty \frac{e^{\beta V(r)}}{r^2 D(r)}\,dr = (4\pi R)^{-1} I(R) \tag{12}$$

where

$$I(r) = r\int_r^\infty \frac{e^{\beta V(r)}}{r^2 D(r)}\,dr. \tag{13}$$

This result [for $D(r) = D$] was first derived by Debye (2).

To generalize the Collins-Kimball association rate in Eq. 6, one must solve Eq. 11 subject to

$$4\pi R^2 D(R) e^{-\beta V(R)}[e^{\beta V(R)} c(R)]' = \kappa c(R). \tag{14}$$

The result is (see, for example, references 18 and 19)

$$k_{CKD}^{-1} = (\kappa e^{-\beta V(R)})^{-1} + k_{SD}^{-1} \tag{15}$$

where k_{SD} is given by Eq. 12. We call this association constant, the Collins-Kimball-Debye rate constant. Eq. 15 reduces to Eq. 6 when $V = 0$ and $D(r) = D$.

FIRST PASSAGE TIMES

The theory of first passage times deals with the problem of calculating the average time, $\tau(r)$, required for a particle starting out at r to reach a boundary for the first time. This boundary can be perfectly or partially absorbing and the inverse of the first passage is a pseudo–first-order rate constant. The first passage time is the solution (13) of the equation:

$$e^{\beta V}\nabla \cdot e^{-\beta V}\mathbf{D} \cdot \nabla \tau = -1 \tag{16}$$

where V is the interaction potential between the particle and the sink. Consider a particle confined to a sphere of

radius R_∞ (i.e., the surface at $r = R_\infty$ is reflecting). The surface of a sphere of radius R ($R < R_\infty$) is partially absorbing. The first passage time for the particle starting out at r_0 to be eventually absorbed by the inner sphere can be found by solving Eq. 16 subject to the boundary conditions that

$$\tau'(R_\infty) = 0 \tag{17}$$

and

$$4\pi D(R)R^2\tau'(R) = \kappa\tau(R). \tag{18}$$

The result (13) is

$$\tau(r_0) = \int_R^{r_0} \frac{e^{\beta V(r)}}{r^2 D(r)}\,dr \int_r^{R_\infty} s^2 e^{-\beta V(s)}\,ds + 4\pi(\kappa e^{-\beta V(R)})^{-1}\int_R^{R_\infty} r^2 e^{-\beta V(r)}\,dr. \tag{19}$$

The mean first passage time for an initial equilibrium distribution (13) is

$$\begin{aligned}\tau &= \left(\int_R^{R_\infty} r^2 e^{-\beta V(r)}\,dr\right)^{-1}\int_R^{R_\infty} r_0^2 e^{-\beta V(r_0)}\,\tau(r_0)\,dr_0 \\ &= \left(\int_R^{R_\infty} r^2 e^{-\beta V(r)}\,dr\right)^{-1}\int_R^{R_\infty} \frac{e^{\beta V(r)}}{r^2 D(r)}\,dr\left(\int_r^{R_\infty} s^2 e^{-\beta V(s)}\,ds\right)^2 \\ &\quad + 4\pi(\kappa e^{-\beta V(R)})^{-1}\int_R^{R_\infty} r^2 e^{-\beta V(r)}\,dr.\end{aligned} \tag{20}$$

We are now in a position to establish the connection between the above τ and the Collins-Kimball-Debye bimolecul ar rate constant (Eq. 15). For a potential that goes to zero as $r\to\infty$, τ diverges as the reflecting boundary is moved to infinity (i.e., $R_\infty\to\infty$). It can be shown that τ diverges as

$$\lim_{R_\infty\to\infty} \tau = \frac{4\pi R_\infty^3}{3} k_{CKD}^{-1} \tag{21}$$

where k_{CKD} is given in Eq. 15. Recall that τ describes the behavior of a single particle. The concentration of this particle is $c_0 = V^{-1} = (4\pi R_\infty^3/3)^{-1}$ and thus we have

$$\tau c_0 = k_{CKD}^{-1}. \tag{22}$$

This relation is a generalization of an analogous result obtained by DeLisi (12) for $V = 0$ and $\kappa\to\infty$.

CAPTURE AND ESCAPE PROBABILITIES

Consider a particle that starts out at a distance r from a perfectly or partially absorbing sink of radius R. What is the probability that the particle escapes to infinity? Onsager (14) and Tachiya (15) showed that the escape probability, $\epsilon(r)$, is the solution of the equation

$$\nabla \cdot e^{-\beta V}\mathbf{D} \cdot \nabla\epsilon = 0 \tag{23}$$

subject to the boundary conditions that

$$\epsilon(\infty) = 1 \tag{24}$$

and

$$\epsilon(R) = 0 \tag{25}$$

for a perfectly absorbing spherical sink or

$$4\pi D(R)R^2\epsilon'(R) = \kappa\epsilon(R) \tag{26}$$

for a partially absorbing one. The capture probability, which we denote by $\gamma(r)$, is clearly given by

$$\gamma(r) = 1 - \epsilon(r). \tag{27}$$

For a perfectly absorbing sink, Eq. 23 is easily solved to yield

$$\gamma_{SD}(r) = RI(r)/rI(R) \tag{28}$$

where $I(r)$ is defined in Eq. 13. In analogy to the association rate constants, we call γ_{SD} the Smoluchowski-Debye capture probability. Note that when the system starts out at contact ($r=R$) $\gamma_{SD} = 1$. The Collins-Kimball-Debye capture probability is also readily obtained by solving Eq. 23 subject to the boundary condition of Eq. 26, with the result that

$$\gamma_{CKD}(r) = \kappa R r^{-1} I(r)[\kappa I(R) + 4\pi R e^{\beta V(R)}]^{-1} \tag{29a}$$

$$= \gamma_{SD}(r) k_{CKD} k_{SD}^{-1} \tag{29b}$$

where we have used Eqs. 12, 15, and 28 to go from Eq. 29a to 29b. Eq. 29a was first derived by Monchick (20) using a different method.

From Eqs. 28 and 29 it immediately follows that

$$k_{CKD} = k_{SD}\gamma_{CKD}(R). \tag{30}$$

This relation, which has been noted previously by Schulten and Schulten (19), has a simple physical interpretation. The Collins-Kimball-Debye association rate constant for a partially absorbing sink is just the Smoluchowski-Debye rate constant for a perfectly absorbing sink times the probability that reactants generated at contact actually react.

RELATIONSHIP TO A KINETIC SCHEME

We are now in a position to consider the relationship of the above results to the kinetic scheme of Eq. 7. The effective forward and reverse rate constants and the equilibrium constant corresponding to this scheme have already been given in Eqs. 8, 9, and 10, respectively. Let us now calculate the capture probability within the framework of this scheme. Suppose we start with the encounter complex A···B. Then the probability that this complex reacts to form AB is

$$\gamma = \frac{k_1}{k_1 + k_-}. \tag{31}$$

Now let us try to equate this γ with $\gamma_{CKD}(R)$ of Eq. 29 and equate k_{CKD} of Eq. 15 with k_f of Eq. 8. It is readily seen

that this can be done in a unique way when

$$k_+ \equiv k_{SD} \tag{32}$$

where k_{SD} is given in Eq. 12 and when

$$\kappa e^{-\beta V(R)} \equiv \frac{k_+}{k_-} k_1 = K_{eq} k_1 . \tag{33}$$

Eq. 32 shows that k_+ which is the bimolecular rate constant for forming the encounter complex A···B, can be identified with the Smoluchowski-Debye association constants for a purely diffusion-controlled reaction, as is expected from simple physical considerations. Eq. 33 clarifies the meaning of the Collins-Kimball intrinsic rate constant, κ, by showing that it is proportional to the equilibrium constant for the formation of the encounter complex (i.e., k_+/k_-) times the rate constant for the reaction of the complex to form the product. Equivalently, $\kappa e^{-\beta V(R)}/k_{-1}$, where k_{-1} is the first-order dissociation constant of the product to yield the encounter complex, is just the equilibrium constant.

The above correspondence allows us to obtain an expression for the effective reverse (i.e., dissociation) rate constant. Using Eqs. 32 and 33 in Eq. 9, for k_r we have

$$k_r^{-1} = k_{-1}^{-1} + k_{-1}^{-1} \kappa e^{-\beta V(R)} k_{SD}^{-1} = k_{-1}^{-1} + K_{eq} k_{SD}^{-1}. \tag{34}$$

This result is basically the same as that obtained by Schurr (21) using a different argument. Using Eqs. 12, 27, and 29, we can rewrite Eq. 34 in a more physically appealing form

$$k_r = k_{-1} \epsilon_{CKD}(R). \tag{35}$$

Thus, the effective reverse (dissociation) rate is just the rate that AB dissociates to form the encounter complex times the escape probability.

EQUILIBRIUM CONSTANTS

The equilibrium constant for the reaction in Eq. 7 can be expressed in terms of the partition functions of the reactants and the product (22) as

$$K_{eq} = \frac{(q_{AB}/V)}{(q_A/V)(q_B/V)} \tag{36}$$

where V is the volume of the system. We assume that A and B are described by the Hamiltonian

$$\mathcal{H}_I = \sum_{i=1}^{3} p_i^2/2m_I \qquad I = A, B \tag{37}$$

i.e., they are structureless. Let $U(|\mathbf{r}_A - \mathbf{r}_B|) = U(\mathbf{r}_{AB})$ be the potential that describes the interaction of A and B in the product AB. The physical nature of the product AB is thus defined by this potential. U has the property that $U(\mathbf{r}_{AB}) \to \infty$ as $\mathbf{r}_{AB} \to \infty$. Using relative and center of mass coordinates, we can describe AB by the Hamiltonian

$$\mathcal{H}_{AB} = \sum_{i=1}^{3} (p_i^{CM})^2/2(m_A+m_B) + \sum_{i=1}^{3} (p_i^R)^2/2\mu + U(\mathbf{r}_{AB}) \tag{38}$$

where $\mu = m_A m_B/(m_A+m_B)$. Using the Hamiltonian in Eq. 37 the classical partition function of the reactants is

$$q_I = V(2\pi m_I k_B T/h^2)^{3/2} \qquad I = A, B. \tag{39}$$

In the usual approach to calculating q_{AB}, one approximates q_{AB} as a product of translational, vibrational, and rotational partition functions, and thus ignores rotation-vibration coupling. This is done primarily because one wishes to treat vibrations quantum mechanically. However, within the framework of classical statistical mechanics, such decomposition is unnecessary and one can obtain a simple exact expression for the equilibrium constant as follows. Using the Hamiltonian in Eq. 38, we have

$$q_{AB} = V[2\pi(m_A+m_B)k_B T/h^2]^{3/2} \cdot (2\pi\mu k_B T/h^2)^{3/2} \int e^{-\beta U(r_{AB})} d\mathbf{r}_{AB}. \tag{40}$$

Using Eq. 36, we immediately have

$$K_{eq} = \int e^{-\beta U(r_{AB})} d\mathbf{r}_{AB}. \tag{41}$$

Thus, the equilibrium constant is simply the Boltzmann factor-weighted volume of the product. In the special case that U is spherically symmetrical, Eq. 41 becomes

$$K_{eq} = 4\pi \int_0^\infty r^2 e^{-\beta U(r)}\, dr. \tag{42}$$

To determine K_{eq} one must define the nature of the product by choosing a specific form for U. For example, we can have $U(r) = U(R) + (1/2)\, U_0(r - R)^2$. Alternately one can choose

$$\begin{aligned} U(r) &= V(R) \qquad r \le R \\ &= \infty \qquad \text{otherwise}, \end{aligned} \tag{43}$$

which yields

$$K_{eq} = \frac{4\pi R^3}{3} e^{-\beta V(R)}. \tag{44}$$

This is precisely the equilibrium constant that is found by dividing the association rate constant, k_{SD}, (Eq. 12) by a diffusive dissociation constant, k_{-SD}, obtained by solving the steady-state Smoluchowski equation (Eq. 11) subject to the boundary condition that $c(\infty) = 0$ and $c(R) = (4\pi R^3/3)^{-1}$ (23). This derivation is difficult to generalize to more complicated geometries because it is unclear what value one should use for c at contact. Eq. 41, the expression for the equilibrium constant, can be used for more complicated geometries once the potential defining the product is specified.

UNIFICATION OF THE WORKS OF DEBYE, KRAMERS, AND COLLINS AND KIMBALL

The Smoluchowski-Debye association rate constant in Eq. 12 was obtained by imposing a completely absorptive boundary condition at $r = R$ (Eq. 1). Eq. 12 is valid for a potential of mean force or arbitrary complexity (e.g.,

multiple barriers). In particular, consider a potential of the form shown in Fig. 1. The reciprocal of the Smoluchowski-Debye rate constant $k_{SD}^{-1}(R)$ (the R in parenthesis serves as a reminder that $c(R) = 0$) is (see Eq. 12)

$$k_{SD}^{-1}(R) = (4\pi)^{-1} \int_R^\infty \frac{e^{\beta V(r)}}{r^2 D(r)} dr. \quad (45)$$

Let us break up the integral from R to ∞ into two parts as follows:

$$k_{SD}^{-1}(R) = (4\pi)^{-1} \int_R^{R'} \frac{e^{\beta V(r)}}{r^2 D(r)} dr + (4\pi)^{-1} \int_{R'}^\infty \frac{e^{\beta V(r)}}{r^2 D(r)} dr. \quad (46)$$

The second term in Eq. 46 is just $k_{SD}^{-1}(R')$. Now let us consider the calculation of the association rate from another point of view. If we assume that the well in Fig. 1 at $r = R$ corresponds to AB while the well at $r = R'$ corresponds to the encounter complex A···B, then we can calculate the association rate using the Collins-Kimball approach by imposing the radiation (partially reflecting) boundary condition at $r = R'$. From Eq. 15, we have

$$k_{CKD}^{-1}(R') = [\kappa e^{-\beta V(R')}]^{-1} + (4\pi)^{-1} \int_{R'}^\infty \frac{e^{\beta V(r)}}{r^2 D(r)} dr. \quad (47)$$

By comparing Eqs. 46 and 47 it can be seen that the results are formally identical [i.e., $k_{SD}^{-1}(R) \equiv k_{CKD}^{-1}(R')$] if

$$\kappa e^{-\beta V(R')} = 4\pi \left(\int_R^{R'} \frac{e^{\beta V(r)}}{r^2 D(r)} dr \right)^{-1}. \quad (48)$$

Thus, for an appropriate choice of the interaction potential the Smoluchowski-Debye and Collins-Kimball-Debye treatments can be made equivalent. Moreover, we will show below that the resulting expression for the Collins-Kimball intrinsic rate constant, κ, (i.e., Eq. 48) is identical to that obtained using Kramers' theory of diffusive barrier crossing.

Before doing this, we note that an analogous correspondence exists between the Smoluchowski-Debye and Collins-Kimball-Debye capture probabilities. Consider a particle generated at $r = R'$ (see Fig. 1). The Smoluchowski-Debye capture probability (capture at $r = R$) is given by Eq. 28, i.e.,

$$\gamma_{SD}(R') = RI(R')/R'I(R) \quad (49)$$

where $I(r)$ is defined in Eq. 13. In the Collins-Kimball treatment, when the radiation boundary condition is imposed at $r = R'$, the analogous capture probability (capture at $r = R'$) can be obtained from Eq. 29 by replacing R by R' and setting $r = R'$,

$$\gamma_{CKD}(R') = \kappa I(R')[\kappa I(R') + 4\pi R' e^{\beta V(R')}]^{-1}. \quad (50)$$

It is easy to show that

$$\gamma_{SD}(R') = \gamma_{CKD}(R') \quad (51)$$

when κ is given by Eq. 48.

We now show that Eq. 48 can be derived from Kramers' theory. Our starting point is Eq. 33, which relates κ to the rate constants of the kinetic scheme in Eq. 7, i.e.,

$$\kappa e^{-\beta V(R')} = \frac{k_+}{k_-} k_1. \quad (52)$$

We have replaced R in Eq. 33 by R' because in this section we are imposing the radiation boundary condition at $r = R'$. The idea of the following demonstration is to derive an expression for k_1 using Kramers' theory, and then calculate the equilibrium constant k_+/k_- using the results of the previous section and then finally show that the κ obtained from Eq. 52 is the same as that given in Eq. 48. Consider the potential shown in Fig. 2. If the system is initially at equilibrium in the well at $r = R'$ and the barrier at R^* is

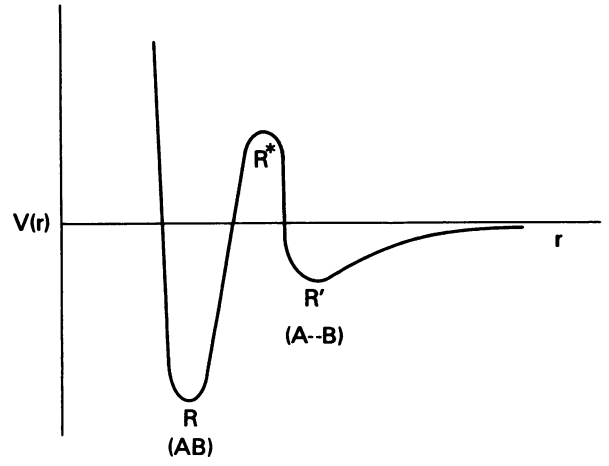

FIGURE 1 The potential used in the text to show the formal equivalence of the Smoluchowski-Debye and the Collins-Kimball-Debye treatments.

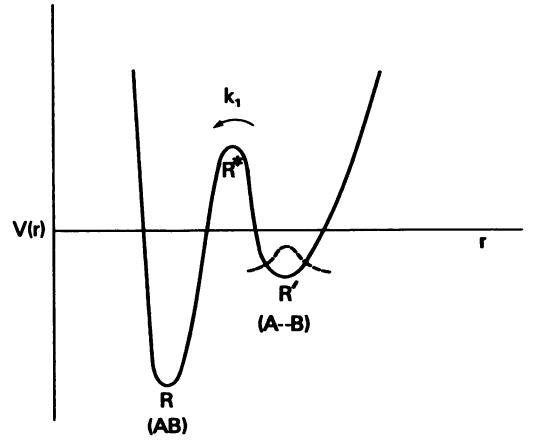

FIGURE 2 The potential for which the first-order rate constant k_1 is calculated in the text using Kramers' theory of diffusive barrier crossing. Because this potential is used to define the complex A···B, it goes to infinity as $r \to \infty$.

larger than $k_B T$ then

$$(k_1^{-1})_K = \int_{R^*}^{\infty} r^2 e^{-\beta V(r)}\, dr \int_R^{R'} \frac{e^{\beta V(r)}}{r^2 D(r)}\, dr. \qquad (53)$$

This equation is the generalization of Kramers' one-dimensional result to three-dimensional spherical geometry (24). If in Eq. 53 one leaves out the Jacobian factors $r^{\pm 2}$, sets $D(r) = D$ and evaluates the first integral by expanding $V(r)$ about $r = R'$ and the second integral by expanding $V(r)$ about $r = R^*$, one recovers Kramers' expression for the rate of diffusive barrier crossing. The equilibrium constant for the formation of the encounter complex A···B can be obtained from Eq. 42. For a high barrier at R^* we have

$$\frac{k_+}{k_-} = 4\pi \int_{R^*}^{\infty} r^2 e^{-\beta V(r)} dr. \qquad (54)$$

Using Eqs. 53 and 54 in Eq. 52 we recover Eq. 48. This completes our demonstration. Thus, the Smoluchowski-Debye treatment is equivalent to the Collins-Kimball-Debye treatment with κ calculated from Kramers' theory.

MULTIPLE BINDING SITES

Competitive Effects

As a final topic, we consider the competitive binding of ligand to a large spherical cell that contains localized receptors uniformly distributed on its surface (see Fig. 3). The kinetics of such reactions have been studied previously by Berg and Purcell (8) and by DeLisi and Wiegel (17) using different methods. At first sight, it might appear that this problem is unrelated to the previous topics of this paper. In fact, it can be handled as a simple application of the previous development.

We treat a spherical cell that is partially covered with reacting sites as a partially reacting sphere. That is, we use the Collins-Kimball theory. Let us consider a spherical cell of radius R that contains N reactive sites on its surface and assume for the moment that there is no long range interaction between the ligands and the cell [i.e., $V(r) = 0$].

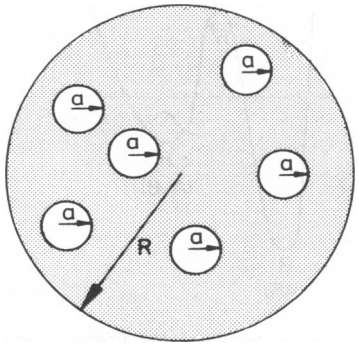

FIGURE 3 Schematic representation of a spherical cell of radius R with reactive sites of radius a uniformly distributed on its surface.

To obtain the association rate we use Eq. 6 with the Collins-Kimball intrinsic rate constant

$$\kappa = N\kappa_0 \qquad (55)$$

where κ_0 is the intrinsic constant for a single site. In this way, we find that

$$k_f = \frac{4\pi DRN\kappa_0}{4\pi DR + N\kappa_0}. \qquad (56)$$

Note that if $4\pi DR >> N\kappa_0$, k_f is proportional to the number of receptors. This limit is realized when the binding to a single receptor is not controlled by diffusion. When the receptors are relatively far apart and the binding to a single receptor is limited by diffusion, then one can approximate κ_0 by the diffusion controlled association constant to a site of radius a lying on an inert surface, i.e.,

$$\kappa_0 = 4Da \qquad (57)$$

where we have used Eq. 3. Substituting Eq. 57 into Eq. 56 we find

$$k_f = \frac{4\pi DRNa}{\pi R + Na}. \qquad (58)$$

This agrees with the result of Berg and Purcell (8), which was obtained in an entirely different way. Eq. 58 shows that k_f is $< 4NDa$. This is a reflection of the fact that the receptors compete for ligand. When $Na >> \pi R$ then $k_f = 4\pi DR$. That is, the cell behaves as if its entire surface is covered by receptors in this limit. As stressed by Berg and Purcell (8), Na can be much greater than πR even when a relatively small fraction of the surface area of the cell is covered by receptors. As we pointed out previously (see the discussion after Eq. 3, and reference 7) this interesting property of diffusion-controlled reactions already appears for a single reactive site on a macromolecule.

Eq. 58 is readily generalized to incorporate a long range (e.g., Coulomb) interaction between the cell and the ligands. Using Eqs. 12, 55, and 57 in Eq. 15, we have

$$k_f^{-1} = (4NDae^{-\beta V(R)})^{-1} + (4\pi)^{-1} \int_R^{\infty} \frac{e^{\beta V(r)}}{r^2 D(r)}\, dr \qquad (59)$$

which is identical to the result of DeLisi and Wiegel (17).

Finally, let us consider the problem of calculating the equilibrium constant. If the binding to the various receptors is not cooperative, then the equilibrium constant for the binding of a ligand to a cell with N identical receptors is just N times the equilibrium constant for binding to a single receptor. Using Eq. 41, we have

$$K_{eq} = N \int e^{-\beta U(\mathbf{r})}\, d\mathbf{r} \qquad (60)$$

where $U(\mathbf{r})$ defines the nature of the stable ligand-receptor complex. For the model shown in Fig. 3, a simple choice is

$$U(r) = V(R) \qquad r \le a$$
$$= \infty \qquad \text{otherwise} \qquad (61)$$

where $V(R)$ is the potential at the surface of the cell, with the result that $K_{eq} = 4\pi Na^3 e^{-\beta V(R)}/3$.

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REFERENCES

1. Smoluchowski, M. V. 1917. Versuch einer mathematischen theorie der koagulationskinetik kolloider lösungen. *Z. Phys. Chem.* 92:129–168.
2. Debye, P. 1942. Reaction rates in ionic solutions. *Trans. Electrochem. Soc.*. 82:265–272.
3. Solc, K., and W. H. Stockmayer. 1971. Kinetics of diffusion-controlled reaction between chemically asymmetric molecules. I. General theory *J. Chem. Phys.* 54:2981–2988.
4. Solc, K., and W. H. Stockmayer. 1973. Kinetics of diffusion-controlled reaction between chemically asymmetric molecules. II. Approximate steady-state solution. *Int. J. Chem. Kinet.* 5:733–752.
5. Schmitz, K. S., and J. M. Schurr. 1972. The role of orientation constraints and rotational diffusion in biomolecular solution kinetics. *J. Phys. Chem.* 76:534–545.
6. Hill, T. H. 1975. Effect of rotation on the diffusion-controlled rate of ligand-protein association. *Proc. Natl. Acad. Sci. U. S. A.* 72:4918–4922.
7. Shoup, D., G. Lipari, and A. Szabo. 1981. Diffusion-controlled bimolecular reaction rates. The effect of rotational diffusion and orientation constraints. *Biophys. J.* 36:697–714.
8. Berg, H. C., and E. M. Purcell. 1977. Physics of chemoreception. *Biophys. J.* 20:193–219.
9. Collins, F. C., and G. E. Kimball. 1949. Diffusion-controlled reaction rates. *J. Colloid Sci.* 4:425–437.
10. Wilemski, G., and M. Fixman. 1972. General theory of diffusion-controlled reactions. *J. Chem. Phys.* 58:4009–4019.
11. Eigen, M. 1974. Diffusion control in biochemical reactions. In Quantum Statistical Mechanics in the Natural Sciences. S. L. Minz and S. M. Wiedermayer, editors. Plenum Publishing Corporation, New York. 37–61.
12. DeLisi, C. 1980. The biophysics of ligand-receptor interactions. *Q. Rev. Biophys.* 13:201–230.
13. Szabo, A. K. Schulten, and Z. Schulten. 1980. First passage time approach to diffusion controlled reactions. *J. Chem. Phys.* 72:4350–4357.
14. Onsager, L. 1938. Initial recombination of ions. *Phys. Rev.* 54:554–557.
15. Tachiya, M. 1978. General method for calculating the escape probability in diffusion-controlled reactions. *J. Chem. Phys.* 69:2375–2376.
16. Kramers, H. A. 1940. Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica.* 7:284–304.
17. DeLisi, C., and F. W. Wiegel. 1981. Effect of nonspecific forces and finite receptor number on rate constants of ligand-cell bound-receptor interactions. *Proc. Natl. Acad. Sci. U. S. A.* 78:5569–5572.
18. Berry, R. S., S. A. Rice, and J. Ross. 1980. Physical Chemistry. John Wiley & Sons, Inc. New York. 1162.
19. Schulten, Z., and K. Schulten. 1977. The generation, diffusion, spin motion, and recombination of radical pairs in solution in the nanosecond time domain. *J. Chem. Phys.* 66:4616–4634.
20. Monchick, L. 1956. Note on the theory of diffusion controlled reactions: application to photodissociation in solution. *J. Chem. Phys.* 24:381–385.
21. Schurr, J. M. 1970. The role of diffusion in bimolecular solution kinetics. *Biophys. J.* 10:700–716.
22. McQuarrie, D. A. 1976. Statistical Mechanics. Harper & Row, Publishers, Inc. New York. 142–159.
23. Hammes, G. G. Principles of Chemical Kinetics. Academic Press, Inc. New York. 66.
24. Brinkman, H. C. 1956. Brownian motion in a field of force and the diffusion theory of chemical reactions. II. *Physica.* 22:149–155.